BIOSYNTHESIS OF PROTEIN A IN STAPHYLOCOCCUS AUREUS

by
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This thesis is a summary of the following publications:

I. Localization of protein A in the bacteria.
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Eur.J.Biochem. 30, 190-194 (1972).

II. A study on the biosynthesis of protein A in *Staphylococcus aureus*.
Movitz, J.
Eur.J.Biochem. 48, 131-136 (1974).

III. Formation of extracellular protein A by *Staphylococcus aureus*.
Movitz, J.
Eur.J.Biochem. 68, 291-299 (1976).

IV. Extracellular protein A from a methicillin resistant strain of *Staphylococcus aureus*.
Lindmark, R., Movitz, J. and Sjöquist, J.

V. Staphylococcal protein A from a set of mutants.
Movitz, J., Masuda, S. and Sjöquist, J.

In the following discussion the papers will be referred to by Roman numerals.

INTRODUCTION

The cell wall of gram-postitive bacteria is a 20-80 nm thick structure, which surrounds the thin plasma membrane (1). An important function of the rigid cell wall is to protect the bacteria and maintain its characteristic shape, but other biological activities are also associated with this structure. Treatment of bacteria with suitable lytic enzymes may completely solubilize the cell wall. The so-formed protoplasts are stable if kept in an hypertonic solution, and retain many of their metabolical activities. Pure cell walls can also be isolated after disintegration of bacteria, followed by differential centrifugations.

The staphylococcal cell wall is mainly built up of three polymers - peptidoglycan, teichoic acid and protein A. The structure of peptidoglycan has been studied in detail (1) and is shown in Fig. 1. Multiple monolayers of peptidoglycan, cross-linked by pentaglycine bridges, are mainly responsible for the shape and integrity of the bacteria. Although the peptidoglycan network of *S.aureus* is remarkably tight, extracellular toxins and enzymes can still penetrate the cell wall during their secretion. The structure of teichoic acid has also been extensively studied (2) and is schematically shown in Fig. 2. This polymer is covalently linked to N-acetylmuramic acid residues of the peptidoglycan by phosphodiester bonds (3). One function of the negatively charged teichoic acid is to maintain the correct environment for the cation-dependent membrane, by binding Mg^{2+} (4). In addition, teichoic

acid is also important for the bacteriophage adsorption in *S.aureus* (5).

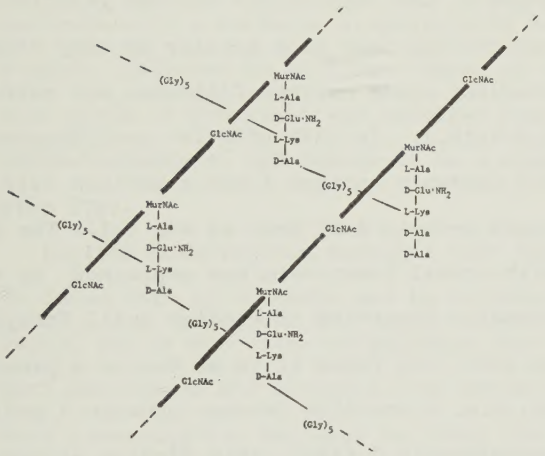

Fig. 1. The peptidoglycan of Staphylococcus aureus. GlcNAc, N-acetyl-D-glucosamine; MurNAc, N-acetylmuramic acid (1).

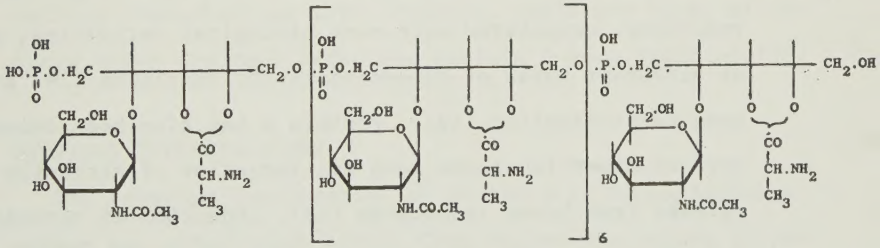

Fig. 2. The teichoic acid of Staphylococcus aureus (wall). Ribitol units are joined through phosphodiester linkages; a N-acteyl-D-glucosamine and a D-alanine residue are linked to every ribitol unit (2).

In 1959 Jensen found that extracts from 75% of all tested strains of *S.aureus* were able to precipitate normal human serum (6). He claimed that a polysaccaride of the cell wall, antigen A, was responsible for the reaction. Similar observations had been made earlier by many other workers when studying crude culture filtrates and extracts of *S.aureus* (7,8,9,10,). In 1962 Löfkvist and Sjöquist established that Jensen's antigen A was a protein (11) and it was later named protein A by Grov *et al.* (12). The reaction of protein A with normal human sera was explained by the existence of normally occurring antibodies until Forsgren and Sjöquist in 1966 (13) found it to be due to a pseudo-immune reaction, *i.e.* a reaction between protein A and the Fc-part of immunoglobulin G (IgG). This finding allowed for easy explanation of many earlier observations. Protein A has since been shown to react with the Fc-part of IgG from most mammalian species (14,15,16,17). The interaction of protein A with the Fc-part of IgG is, as with normal antigen-antibody reactions, associated with many biological activities, such as different types of hypersensitivity reactions (14) and complement fixation (18). Protein A has also been shown to perform other functions such as: induction of histamine release from human leucocytes (19), induction of mitosis in B-lymphocytes (20), neutralization of virus-IgG complexes (21) and inhibition of the adsorption of phages to the surface of *S.aureus* (22).

Previously, in isolating protein A by means of heat extractio

of bacteria (6), an heterogeneous material of molcular weight 15,000 resulted (23). Homogeneous protein A (24) of molecular weight 42,000 (25) could be isolated after digestion of the bacteria with a suitable staphylolytic enzyme such as lysostaphin, followed by different types of chromatography. A high yield of protein A was obtained when using affinity chromatography on IgG-Sepharose as a single purification step (26).

Earlier observations revealed that IgG-reacting activity is found both in extracts and in culture-filtrates of *S.aureus* (6,9), and it was later established that the bacteria produce both cell-bound and extracellular protein A (27). The aim of this investigation has been to study the localization of cell-bound protein A in the bacteria (I) and its incorporation into the cell wall (II). Furthermore it was of interest to investigate the nature and origin of extracellular protein A (III). Protein A from a methicillin resistant strain was also isolated and characterized (IV). Finally, different types of mutants which produced defective protein A were isolated (V).

PREVIOUS INVESTIGATIONS

The exterior localization of protein A in *Staphylococcus aureus* was established early. Even before the nature of protein A was known, it was observed that normal human sera agglutinate the bacteria (6). Use of different microscopy techniques, such as electron microscopy of sectioned bacteria (28,29,30) or immunofluorescence microscopy (31), has enabled

many workers to study the reaction between normal IgG and protein A on the surface of *S.aureus*. The ability of the bacteria to bind IgG was destroyed after digestion with trypsin (29,30). It was shown by Forsgren that protein A could be removed from the bacteria by lysozyme digestion, without any noticeable release of intracellular material (27). Moreover, only small amounts of protein A could be detected in the cytoplasm. Protein A has also been detected in, or isolated from, purified cell walls of *S.aureus* (32,33). By analyzing fractionated cell walls, Grov and Rude suggested that protein A was loosely attached to the peptidoglycan moiety (34). *S.aueus* was observed to produce extracellular, as well as cell-bound protein A, and Forsgren found that 30% of all protein A produced by exponentially growing bacteria was released into the culture medium (27). Certain methicillin resistant strains cannot incorporate protein A into the cell wall and therefore all protein A becomes extracellular (35,36).

The cell wall of gram-positive bacteria contains two main polymers, peptidoglycan and teichoic acid, the biosynthesis of which has been extensively studied (37,4). Due to the localization of the cell wall outside the cytoplasmic membrane, some steps in the biosynthesis take place in the cytoplasm and some either in contact with, or outside, the membrane. Peptidoglycan precursors are made on a uradylic acid carrier in the cytoplasm and are then transferred to a membrane-bound carrier. The precursors are added to the growing peptidoglycan chains after certain substitutions. The nascent peptidoglycan

is finally cross-linked by transpeptidation, in order to make it insoluble. Teichoic acid precursors are also synthesized in the cytoplasm, but they are linked to a cytidylic acid carrier. The precursors are transferred to some membrane-bound carrier and then added to growing teichoic acid chains. It is known that teichoic acid chains are covalently linked to the peptidoglycan of the cell wall (3). Mauck and Glaser studied the assembly of the cell wall in *Bacillus subtilis* and found that teichoic acid was only linked to newly synthesized peptidoglycan chains (38).

The cell envelope of *Escherichia coli* contains a small lipoprotein, the structure and biosynthesis of which is now well-known (39). The lipoprotein is built from 58 amino acids, forming repetitive sequences. Its N-terminus binds three fatty acids and its C-terminus is covalently linked to the diaminopimelic acid residue of every 10th-12th peptidoglycan monomer. One bacteria contains approximately 750,000 lipoprotein molecules, of which one third are bound to the peptidoglycan and two thirds are free in the periplasm. The molecules are linked to presynthesized peptidoglycan, probably by a penicillin-insensitive transpeptidation reaction. This is in contrast to teichoic acid of gram-positive bacteria.

Protein A isolated after lysostaphin digestion of strain Cowan I bacteria, has a molecular weight of 42,000 (25). Physicochemical analyses revealed a frictional ratio of 2.1-2.2 and an intrinsic viscosity of 29 ml/g suggesting a markedly extended shape for the molecule (25). Moreover, the

content of α-helix was estimated to be approximately 50% (40). Lysine was shown to be the carboxyterminal amino acid, by means of digestion with carboxypeptidase A and B. However, an extraordinarily long digestion time had to be used (24). The Edman technique indicated a blocked amino-terminus, but the nature of this phenomenon was not established (24). Nitration of the tyrosine residues of protein A efficiently reduces its Fc-reactivity, indicating that the tyrosines may play a role in the protein A-Fc reaction (41). Based on the observation that one molecule of protein A can bind two IgG molecules, Sjöquist *et al.* (24) suggested that protein A has at least two identical Fc-binding structures. The structure of protein A in relation to its reaction with IgG has recently become clear. After partial tryptic digestion of intact protein A, fragments of a molecular weight of approximately 7,000 were isolated (42). The fragments were monovalent in their reaction with IgG, and showed striking similarities in terms of amino acid composition and peptide maps. The molar yield of the monovalent fragments after digestion was 210% of the amount of protein A used, indicating at least three active site regions in protein A. Sjödahl later showed that protein A contains four repetitive sequences of molecular weight 7,000 (43). Each of the four structures is able to react with IgG, and they are located close together in the N-terminal section of the protein A molecule. The remaining C-terminal section of the molecule, comprising about 150 residues, has no IgG-binding property, but is suggested to be linked to the cell wall in an unknown

manner.

The selective secretion of extracellular enzymes and toxins is a common process in many cells. In eucaryotic cells it is suggested that ribosomes bound to the endoplasmatic reticulum synthesize the extracellular proteins (44). However, bacterial secretion of proteins has been less extensively investigated. Extracellular enzymes and toxins are relatively small molecules and contain few or no disulphide bridges, which probably facilitates the passage of the proteins through membrane and cell wall (45). It has been postulated in many reports that release of extracellular enzymes depends on a *de novo* synthesis (46,47). The enzymes are secreted directly after synthesis on the ribosomes, and no significant pool of preformed enzyme was detected in the cytoplasm (47). Cancedda and Schlesinger showed that alkaline phosphatase, an extracellular enzyme of *E.coli*, is preferentially synthesized on membrane-bound ribosomes (48). It has been hypothesized by Both *et al.* that the synthesis of extracellular enzymes occurs close to the membrane at special translational-extrusion sites (49). The extended polypeptide chain is extruded through the membrane and then assumes its active conformation. The cell wall is seldom necessary for the synthesis of extracellular enzymes and it has been shown that protoplasts of *S.aureus* can also be induced to synthesize extracellular penicillinase (50).

Protein A mutants of *S.aureus* have been isolated by Forsgren *et al.* after exposure of the bacteria to different kinds of mutagens (51). The mutants were protein A deficient or had

decreased protein A production. Most mutants were also deficient in many extracellular enzymes and toxins. Spontaneous or induced revertants often regained all lost properties, suggesting the existence of a common control mechanism for the phenotypic expression of those properties.

Masuda *et al.* (52) used a cosedimentation technique to select mutants which were deficient in cell-bound protein A. The isolated mutants were of two types, one of which was protein A deficient (HL-mutants), while the other produced large amounts of extracellular protein A (LH-mutants). It was also observed that protein A from LH-mutants had a lower molecular weight than that from the parental strain.

PRESENT INVESTIGATIONS

The localization of protein A (I)

Previous reports have indicated that protein A is a cell wall constituent of Staphylococcus aureus. However, the manner in which protein A is linked to the cell wall has never been established.

Protein A was quantified in lyophilized strain Cowan I bacteria and in isolated cell walls, by using a single radial immunodiffusion technique. It was found that the bacteria contain 1.7% protein A and the cell walls 6.7%, indicating that protein A is mainly located in the cell wall. Extraction of cell walls with detergents or hot trichloroacetic acid (TCA) in order to remove membrane fragments and teichoic acid respectively, did not liberate any protein A. This indicates that protein A is

linked to the peptidoglycan moiety of the cell wall. A decrease of protein A activity was observed in the cell walls after the extraction with hot TCA. However, TCA was shown to have a similar effect on isolated protein A and it was thus concluded that the reduction of protein A in TCA-extracted cell walls was due to an inactivation of protein A rather than to a release. High concentrations of LiCl have been used to solubilize proteins which are strongly, but not covalently, bound to the cell wall. Cell walls of *S.aureus* were extracted with up to 10 molar solutions of LiCl but no protein A was released, suggesting that protein A is covalently linked to the cell wall.

The staphylococcal cell wall is a poor substrate for hen egg white lysozyme. However, digestion of *S.aureus* with lysozyme in a high enzyme : substrate ratio slowly liberates protein A. Protein A isolated after such a lysozyme digestion appeared to be heterogeneous and two bands were observed after disc-electrophoresis in polyacrylamide gels. Chromatography of such a protein A preparation on Sephadex G-100 resulted in two fractions (I and II) which were subjected to amino acid analyses. The amino acid and hexosamine composition of fractions I and II appeared to be very similar. However, fraction I contained additional residues of lysine, glutamic acid, proline, alanine, glycine, muramic acid and glucosamine, *i.e.* mainly the constituents of peptidoglycan. Fraction I was digested with lysostaphin, which contains three enzymatic activities acting on staphylococcal peptidoglycan. The digestion products were separated on Sephadex G-50. The second and third peaks of the chromatogram,

comprising material of low molecular weight, were finally subjected to amino acid and hexosamine analyses. The result indicated that both peaks had the same composition as peptidoglycan. However, the third peak was devoid of hexosamines. Therefore it was concluded that protein A, isolated after lysozyme digestion, is covalently linked to a peptidoglycan fragment, and consequently that protein A in the cell wall is covalently linked to the peptidoglycan moiety.

The cell wall of strain Cowan I contains 6.7% protein A. After solubilization of teichoic acid in the cells walls, by hot TCA extraction, a 30% reduction of the cell wall weight was observed. Apparently the cell wall contains 63% peptidoglycan, thus it was calculated that one molecule of protein A is linked to every 325th peptidoglycan monomer.

Affinity chromatography for the study of the biosynthesis of protein A (II, III and additional results)

Protein A reacts with the Fc-part of human IgG (13). This property was utilized to isolate protein A by means of affinity chromatography on Sepharose 4B to which IgG was covalently linked (26). The specificity of the affinity chromatography makes this method also suitable for studying the biosynthesis of protein A.

Radioactively labelled protein A can be rapidly isolated from bacteria grown in the presence of a labelled amino acid. The synthesis of protein A is then quantified by counting the radioactivity of the isolated material. Extracellular protein A

was determined in cell-free culture broth and cell-bound protein A after digesting bacteria with lysostaphin. The samples were applied on columns containing IgG-Sepharose; and after washing the columns with neutral buffer, protein A was eluted with a low pH buffer. A buffer of pH 3.0 was found to be best to elute protein A from the columns. Owing to the high capacity of IgG-Sepharose, columns containing 0.2 ml gel were sufficient for a wide range of samples. Fig. 3 shows that the recovery of radioactivity is directly proportional to the size of the sample which was applied on the column.

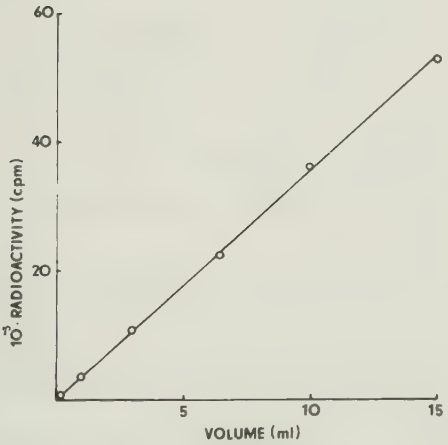

Fig. 3. Affinity chromatography of radioactively labelled protein A. Bacteria of strain Cowan I were cultivated overnight in the presence of ^{3}H-lysine (1μCi/ml). The suspension was digested with lysostaphin and centrifuged. Protein A was isolated from various volumes of the supernatant by affinity chromatography on 0.2 ml IgG-Sepharose (III).

Biosynthesis of cell-bound protein A (II)

In strain Cowan I most protein A is covalently linked to the peptidoglycan moiety of the cell wall. In order to understand the assembly of the staphylococcal cell wall it has been necessary to study the incorporation of protein A into the cell wall, in relation to the incorporation of new peptidoglycan and teichoic acid chains.

Antibiotics, such as chloramphenicol, streptomycin and puromycin inhibit protein synthesis by affecting different functions of the ribosome. These antibiotics were also shown to inhibit the *de novo* synthesis of protein A, but peptidoglycan synthesis continues for about one generation. On the other hand vancomycin effectively inhibits the formation of peptidoglycan without affecting the *de novo* synthesis of protein A. The *de novo* synthesis of peptidoglycan and of protein A are thus not dependent on each other.

Vancomycin is able to inhibit the addition of new peptidoglycan monomers to growing peptidoglycan chains, by binding to free D-ala-D-ala residues of the chains. However, another experiment has shown that incorporation of protein A into the cell wall continues in the presence of vancomycin. On the other hand, incoporation of tecihoic acid is completely inhibited. It has been shown that teichoic acid can be only linked to peptidoglycan chains synthesized at the same time as the former polymer (38). Thus it was concluded that protein A can be linked to pre-existing peptidoglycan chains, while teichoic acid cannot.

Although the incorporation of protein A into the cell wall continues in the presence of vancomycin, the rate of incorporation is lower than normal. There are two explanations for this observation- either the large, bulky vancomycin molecule, bound to the D-ala-D-ala residues of the peptidoglycan, disturbes the incorporation of protein A by sterical hindrance or vancomycin competes with protein A for the same binding site on the peptidoglycan.

Pulse-labelling experiments show that protein A is rapidly incorporated into the cell wall after synthesis on the ribosomes. The reaction is complete within one minute, indicating that protein A, which is found both in the cytoplasm and in the culture medium, is probably not precursor for the cell wall protein A. The incorporation of protein A is thus dependent on a *de novo* synthesis, as with the release of many extracellular enzymes (46, 47). On the other hand, the lipoprotein of E.coli exists both free in the periplasm and linked to the peptidoglycan, and the free form is the precursor of the bound form (53).

The biosynthesis of extracellular protein A (III)

Most strains of *S.aureus* produce cell-bound as well as extracellular protein A, as reported by Forsgren (27). These results were also confirmed in the present investigation, using another technique. The release of protein A was observed in both the exponential and stationary growth phases. In exponentially growing strain Cowan I bacteria, extracellular protein A comprises approximately 8% of the total amount of protein A. However, a great day-to-day variation in the production of extracellular protein

A was observed, which is difficult to explain. As indicated above, less protein A is secreted from exponentially growing bacteria than previously reported (27). This is explained by the fact that different methods were used to quantify protein A. Moreover, the yield from a preparation of extracellular protein A is low and corresponds to a release of about 8%.

Pulse-labelling experiments show that in exponentially growing bacteria, most extracellular protein A is secreted directly after synthesis on the ribosomes, without passing a cell wall-bound state. Only approximately 1.5% of the protein A secretion is not dependent on a *de novo* synthesis and this protein A must originate from a pre-synthesized pool. No *de novo* synthesis of protein A occurs in stationary bacteria, and all release of protein A is due to cell wall turn over.

Cell-bound and extracellular protein A were isolated from three strains. The two types of protein A were characterized by electrophoresis in polyacrylamide gels with and without SDS and by amino acid analysis, but no differences were noticed within each strain. This strongly suggested that the two types of protein A were synthesized with the same messenger RNA as template.

It was possible to increase the production of extracellular protein A by manipulation of both the acceptor for cell-bound protein A, *i.e.* the cell wall, and the protein A molecule itself. Formation of protoplasts was induced by the action of

lysostaphin in 0.7 M sucrose. The protoplasts were completely devoid of cell wall but retained their ability to synthesize protein A as efficiently as the bacteria. Nearly all protein A produced by the protoplasts was found in the culture medium, and only about 1% was found in the cytoplasm. It was shown in another experiment that low concentrations of puromycin enhanced the production of extracellular protein A, simultaneously inhibiting the production of cell-bound protein A. The enhancement was only observed in cells containing cell-bound protein A. Puromycin is known to cause the premature release of polypeptide chains from the ribosomes. In analogy, defective protein A may be produced in the presence of puromycin and thus can not be incorporated into the cell wall. It is tempting to suggest that protein A is normally bound to the cell wall and that the secretion is due to defects in some protein A molecules, in the cell wall or in those mechanisms which are responsible for the incorporation of protein A into the cell wall.

Characterization of protein A from a methicillin resistant strain of S.aureus (IV)

The production of mainly cell-bound protein A is a feature of most strains of *S.aureus*. However, certain methillin-resistant strains produce extracellular protein A only. Such a methicillin-resistant strain, A676, was used for the isolation and characterization of a true extracellular protein A, in order to ascertain any differences between extracellular and cell-bound protein A.

The synthesis of extracellular protein A in strain A676 is coupled to cell growth, no protein A being produced during the stationary growth phase. Pulse-labelling experiments indicated that the secretion is dependent on a *de novo* synthesis of protein A, similar to the secretion of extracellular protein A in strain Cowan I.

Extracellular protein A was isolated from strain A676 with a yield of 33 mg/l culture medium. Thus strain A676 produces as much protein A as strain Cowan I. However, isolation of protein A from strain A676 was more rapid, since prior digestion with lysostaphin was not required. The isolated material appeared to be homogeneous as judged by different types of polyacrylamide disc-electrophoresis and N-terminal analysis.

The molecular weight of A676 protein A was estimated to be 41,000, as measured by gel chromatography on Sepharose 6B in guanidine hydrochloride and equilibrium sedimentation. This value is close to 42,000, which is reported to be the molecular weight of Cowan I protein A (25). However, both types of protein A have apparent higher molecular weights when determined by polyacrylamide disc-electrophoresis in SDS. The isoelectric points for the two types of protein A were estimated to be 5.1. The determinations were performed in cylindrical polyacrylamide gels containing the Ampholines. It was convenient to use radio-iodinated samples of protein A, since pH and protein A could then be determined simulataneously. Amino acid analyses revealed only minor differences in the amino acid composition of protein A from the two strains.

Edman degradation of A676 protein A led to determination of the amino-terminal sequence as Ala-Gln-Asp-Glx-Ala-Lys- while carboxypeptidase digestion revealed a carboxy-terminal sequence of -Val-Ala-Lys. In contrast, the N-terminus of cell-bound protein A from strain Cowan I is blocked,while lysine is the first amino acid which is liberated at a very low rate from the protein by digestion with carboxypeptidases (24). Therefore, this type of protein A may also have a blocked C-terminus.

Mutants of S.aureus producing defective protein A (V)

Six mutants were isolated from strain Cowan I, using the rapid cosedimentation technique. One of the mutants resulted from spontaneous mutation while the others were the products of mild ultraviolet irradiation. Deficiency in cell-bound protein A and increased secretion of extracellular protein A were characteristics of all mutants. Protein A was isolated from the mutants and was found to have molecular weights ranging between 12,800 and 41,000, in contrast to the parental protein A which has a molecular weight of 42,000. This change in size of the mutant proteins may be the reason why they are not incorporated into the cell wall. However, one cannot exclude the possibility that changes, either in cell wall composition or in an enzyme system involved in protein incorporation, caused the secretion of the mutant proteins.

The N-terminal section of parental protein A has four similar active IgG-reacting regions with a known amino acid

sequence (43,54). The amino acid analyses of the mutant protein compared with the amino acid sequences of the N-terminal IgG-reacting fragments of native protein A reveal that all these proteins share a common N-terminal structure. Moreover, it was shown that all mutant proteins, and extracellular protein A from the parental strain, have alanine as the N-terminal amino acid. Sjödahl (43) has shown that the C-terminal part of protein A is linked to the cell wall. It is thus suggested that the shortening of the polypeptide chain towards the C-terminus, which was observed in the mutant proteins, is the reason why these cannot be incorporated into the cell wall.

It has been shown earlier that a nonsense mutation can evoke a shortening of the polypeptide chain *i.e.* a new triplet has arisen by a base pair substitution, causing a premature termination of the polypeptide chain (55). Such a chain termination mutant protein shares the N-terminal amino acid sequence with the parental protein. Owing to the results from the amino acid and N-terminal analyses, it is tempting to suggest that the shortening of the polypeptide chain of the mutant proteins, compared with the parental protein A, is a result of such chain termination mutations.

GENERAL SUMMARY

1. About 90% of the protein A produced by *Staphylococcus aureus*, strain Cowan I, is located in the cell wall. 1% is found in the cytoplasm and 8% is released into the culture medi

2. Protein A in the cell wall is covalently linked to the peptidoglycan moiety.

3. Protein A is either incorporated into the cell wall or released into the culture medium, directly after synthesis on the ribosomes. Therefore, it is suggested that protein A is synthesized on ribosomes located close to the cytoplasmic membrane.

4. The synthesis of protein A and peptidoglycan takes place independently of each other. Protein A can be linked to pre-existing peptidoglycan chains while teichoic acid cannot. Protein A and teichoic acid are thus incorporated into the cell wall by different mechanisms.

5. In exponentially growing bacteria, protein A is secreted into the medium without passing through a cell wall-bound state. Conversely, extracellular protein A is not a precursor of cell wall protein A. Bacteria in the stationary phase synthesize no *de novo* protein A, thus the release of protein A in this situation originates from cell wall turn over.

6. Protoplasts of *S.aureus* synthesize protein A as efficinetly as the bacterial form, but all protein A is secreted. In bacteria, puromycin enhances the production of extracellular protein A and simulataneously inhibits the incorporation of protein A into the cell wall. The enhancement is only observed in bacteria containing cell wall protein A.

7. Extracellular protein A isolated from a methicillin resistant strain, A676, is similar to cell-bound protein A of strain Cowan I in terms of molecular weight, amino acid

composition, isoelectric point and IgG-reactivity. However, A676 protein A has both a free N-terminal and C-terminal amino acid, in contrast to cell-bound protein A from strain Cowan I.

8. Six mutants were isolated which cannot incorporate protein A into the cell wall; all protein A being secreted. The mutants produce protein A of molecular weights ranging between 12,800 and 41,000. It is suggested that the shortening of the polypeptide chains of the mutant proteins is due to chain termination mutations.

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REFERENCES

1. Ghuysen, J.-M. (1968) *Bacteriol.Rev. 32,* 425-464.
2. Baddiley, J. (1962) *Federation Proc. 21,* 1084-1088.
3. Ghuysen, J.-M., Tipper, D.J. & Strominger, J.L. (1965) *Biochemistry 4,* 474-485.
4. Baddiley, J. (1972) *Essays in Biochemistry 8,* 35-77.
5. Coyette, J. & Ghuysen, J.-M. (1968) *Biochemistry 7,* 2385-2389.
6. Jensen, K. (1959) *Thesis,* Munksgaard, Copenhagen.
7. Beiser, S.M., Dworetzky, M., Smart, K.M. & Baldwin, H.S. (1958) *J.Allergy 29,* 44-47.
8. Neter, E., Gorzynski, E.A., Drislane, A.M., Harris, A.H. & Rajnovich, E. (1959) *Proc.Soc.Exp.Biol.Med. 101,* 484-487.
9. Cohen, J.O., Cowart, G.S. & Cherry, W.B. (1961) *J.Bacteriol. 82,* 110-114.
10. Lenhart, N.A., Mudd, S., Yoshida, A. & Li, I.W. (1963) *J.Immunol. 91,* 771-776.
11. Löfkvist, T. & Sjöquist, J. (1962) *Acta Pathol.Microbiol. Scand. 56,* 295-304.
12. Grov, A., Myklestad, B. & Oeding, P. (1964) *Acta Pathol. Microbiol.Scand. 61,* 588-596.
13. Forsgren, A. & Sjöquist, J. (1966) *J.Immunol. 97,* 822-827.
14. Sjöquist, J., Forsgren, A., Gustafson, G.T. & Stålenheim, G. (1967) *Cold Spring Harbor Symp. Quant. Biol. 32,* 577-581.
15. Forsgren, A. & Sjöquist, J. (1967) *J.Immunol. 99,* 19-24.
16. Forsgren, A. (1968) *J.Immunol. 100,* 927-930.
17. Kronvall, G., Seal, U.S., Finstad, J. & Williams, Jr., R.C. (1970) *J.Immunol. 104,* 140-147.

18. Sjöquist, J. & Stålenheim, G. (1969) *J.Immunol. 103,* 467-473

19. Petersson, B.-Å. (1975) *Scand. J. Immunol. 4,* 777-784.

20. Forsgren, A., Svedjelund, A. & Wigzell, H. (1976) *Eur. J. Immunol. 6,* 207-213.

21. Austin, R.M. & Daniels, C.A. (1974) *J.Immunol. 113,* 1568-1574.

22. Nordström, K. & Forsgren, A. (1974) *J. Virol. 14,* 198-202.

23. Forsgren, A. & Sjöquist. J. (1969) *Acta Pathol. Microbiol. Scand. 75,* 466-480.

24. Sjöquist, J., Meloun, B. & Hjelm, H. (1972) *Eur. J. Biochem. 29,*572-578.

25. Björk, I., Petersson, B.-Å. & Sjöquist, J. (1972) *Eur. J. Biochem. 29,*579-584.

26. Hjelm, H., Hjelm, K. & Sjöquist, J. (1972) *FEBS Lett. 28,* 73-76.

27. Forsgren, A. (1969) *Acta Pathol. Microbiol. Scand. 75,*481-490

28. Lind, I., Reyn, A. & Birch-Andersen, A. (1964)*Proc. 3rd Eur. Conf. Electron. Microscop. vol. B,* 535-536.

29. Virgilio, A.R., González, P.C., Munoz, S.N. & Mendoza, G. S. (1970) *Infect. Immun. 2,* 342-344.

30. Nickerson, D.S., White, J.G., Kronvall, G., Williams, Jr., R.C. & Quie, P.G. (1970) *J. Exp. Med. 131,* 1039-1047.

31. Forsgren, A. & Forsum, U. (1970) *Infect. Immun. 2,* 387-391.

32. Yoshida, A., Mudd, S. & Lenhart, N.A. (1963) *J. Immunol. 91,* 777-782.

33. Grov, A. & Rude, S. (1967) *Acta Pathol. Microbiol. Scand. 71,* 409-416.

34. Grov, A. & Rude, S. (1967) *Acta Pathol. Microbiol. Scand. 71,* 417-421.

35. Lind, I. (1972) *Acta Pathol. Microbiol. Scand. Sect. B. 80,* 702-708.

36. Winblad, S. & Ericson, C. (1973) *Acta Pathol. Microbiol. Scand. Sect. B. 81,* 150-156.

37. Ghuysen, J.-M. & Shockman, G.D. (1973) *Bacterial Membranes and Walls* ed. L. Leive, p.p. 37-130. Dekker, New York.

38. Mauck, J. & Glaser, L. (1972) *J.Biol. Chem. 247,* 1180-1187.

39. Braun, V. & Hantke, K. (1974) *Ann. Rev. Biochem. 43,* 89-121.

40. Sjöholm, I. (1975) *Eur. J. Biochem. 51,* 55-61.

41. Sjöholm, I., Bjerkén, A. & Sjöquist, J. (1973) *J. Immunol. 110,* 1562-1569.

42. Hjelm, H., Sjödahl, J. & Sjöquist, J. (1975) *Eur. J. Biochem. 57,* 395-403.

43. Sjödahl, J. (1976) Accepted for publication in *Eur. J. Biochem.*

44. Andrews, T.M. & Tata, J.R. (1971) *Biochem. J. 121,* 683-694.

45. Lampen, J.O. (1965) *Symp. Soc. Gen. Microbiol. 15,* 115-133.

46. Welker, N.E. & Campbell, L.L. (1963) *J. Bacteriol.86,* 1202-1210.

47. May, B.K. & Elliott, W.H. (1968) *Biochim. Biophys. Acta 157,* 607-615.

48. Cancedda, R. & Schlesinger, M.J. (1974) *J. Bacteriol. 117,* 290-301.

49. Both, G.W., McInnes, J.L., Hanlon, J.E., May, B.K. & Elliott, W.H. (1972) *J. Mol. Biol. 67,* 199-217.

50. Coles, N.W. & Gross, R. (1973) *Antimicrob. Ag. Chemother. 4,* 178-185.

51. Forsgren, A., Nordström, K., Philipson, L. & Sjöquist, J. (1971) *J. Bacteriol. 107,* 245-250.

52. Masuda, S., Sakurai, S. & Kondo, I. (1975) *Infect. Immun. 12,* 245-251.

53. Inouye, M., Shaw, J. & Shen, C. (1972) *J. Biol. Chem. 247,* 8154-8159.

54. Sjödahl, J. (1976) in manuscript.

55. Sarabhai, A.S., Stretton, A.O.W., Brenner, S. & Bolle, A. (1964) *Nature, 201,* 13-17.

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